



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(54) Title:</b> HBV AMPLIFIER PROBES FOR USE IN SOLUTION PHASE SANDWICH HYBRIDIZATION ASSAYS  <b>(57) Abstract</b>  Novel DNA probe sequences for detection of HBV in a sample in a solution phase sandwich hybridization assay are described. Amplified nucleic acid hybridization assays using the probes are exemplified.		
<p>Applicants: Chong-Jin Oon, et al. U.S. Serial No.: 09/362,394 Filed: July 28, 1999 (Exhibit 2)</p>		

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HBV AMPLIFIER PROBES FOR USE IN SOLUTION PHASE  
SANDWICH HYBRIDIZATION ASSAYS

10 Technical Field

This invention is in the field of nucleic acid hybridization assays. More specifically, it relates to novel nucleic acid probes for detecting Hepatitis B Virus (HBV).

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Background Art

Viral hepatitis is a systemic disease involving primarily the liver, with HBV being primarily responsible for most cases of serum or long-incubation hepatitis.

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Antigenic characterization of HBV derives from the complex protein found on the virus's surface. One antigenic specificity, designated a, is common to all HBV surface antigen (HBsAg), while two other sets of mutually exclusive determinants result in four principle subtypes of HBsAg: adw, ayw, adr, and ayr.

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Pasek et al. (Nature 282:575-579, 1979) disclosed the entire nucleotide sequence of subtype ayw HBV genomic DNA.

Valenzuela et al. (Animal Virus Genetics, Field et al., eds., Academic press, NY, 1981) reported the complete nucleotide sequence of subtype adw2 HBV DNA.

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EPA Pub. No. 0068719 disclosed the sequence and expression of HBsAg from the adw serotype.

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Fujiyama et al. (Nucleic Acid Research 11:4601-4610, 1983) disclosed the entire nucleotide sequence of serotype adr HBV DNA.

British patent application No. 2034323A,  
5 published 6/4/80, describes the isolation and cloning of the HBV genome and its use to detect HBV in serum.

Berninger et al. (J. Med. Virol. 9:57-68, 1982) discloses an assay based on nucleic acid hybridization which detects and quantitates HBV in serum, using the  
10 complete HBV genome as probe.

U.S. 4,562,159 discloses a method and test kit for the detection of HBV by DNA hybridization using cloned, genomic HBV DNA as a probe.

Commonly owned U.S. 4,868,105 describes a  
15 solution phase nucleic acid sandwich hybridization assay in which analyte nucleic acid is first hybridized in solution to a labeling probe set and to a capturing probe set in a first vessel. The probe-analyte complex is then transferred to a second vessel that contains a solid-  
20 phase-immobilized probe that is substantially complementary to a segment of the capturing probes. The segments hybridize to the immobilized probe, thus removing the complex from solution. Having the analyte in the form of an immobilized complex facilitates  
25 subsequent separation steps in the assay. Ultimately, single stranded segments of the labeling probe set are hybridized to labeled probes, thus permitting the analyte-containing complex to be detected via a signal generated directly or indirectly from the label.

Commonly owned European Patent Application  
30 (EPA) 883096976 discloses a variation in the assay described in U.S. 4,868,105 in which the signal generated by the labeled probes is amplified. The amplification involves the use of nucleic acid multimers. These  
35 multimers are branched polynucleotides that are

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constructed to have a segment that hybridizes specifically to the analyte nucleic acid or to a nucleic acid (branched or linear) that is bound to the analyte and iterations of a second segment that hybridize specifically to the labeled probe. In the assay employing the multimer, the initial steps of hybridizing the analyte to label or amplifier probe sets and capturing probe sets in a first vessel and transferring the complex to another vessel containing immobilized nucleic acid that will hybridize to a segment of the capturing probes are followed. The multimer is then hybridized to the immobilized complex and the labeled probes in turn hybridized to the second segment iterations on the multimer. Since the multimers provide a large number of sites for label probe attachment, the signal is amplified. Amplifier and capture probe sequences are disclosed for Hepatitis B virus, Neisseria gonorrhoeae, penicillin and tetracycline resistance in N. gonorrhoeae, and Chlamydia trachomatis.

Commonly owned copending application Serial No. 558,897, filed 27 July 1990, describes the preparation of large comb-type branched polynucleotide multimers for use in the above-described solution phase assay. The combs provide greater signal enhancement in the assays than the smaller multimers.

#### Disclosure of the Invention

One aspect of the invention is a synthetic oligonucleotide useful as an amplifier probe in a sandwich hybridization assay for HBV comprising a first segment having a nucleotide sequence substantially complementary to a segment of HBV nucleic acid and a second segment having a nucleotide sequence substantially complementary to an oligonucleotide multimer.

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Another aspect of the invention is a synthetic oligonucleotide useful as a capture probe in a sandwich hybridization assay for HBV comprising a first segment having a nucleotide sequence substantially complementary to a segment of HBV nucleic acid and a second segment having a nucleotide sequence substantially complementary to an oligonucleotide bound to a solid phase.

Another aspect of the invention is a solution sandwich hybridization assay for detecting the presence of HBV in a sample, comprising

(a) contacting the sample under hybridizing conditions with an excess of (i) an amplifier probe oligonucleotide comprising a first segment having a nucleotide sequence substantially complementary to a segment of HBV nucleic acid and a second segment having a nucleotide sequence substantially complementary to an oligonucleotide unit of a nucleic acid multimer and (ii) a capture probe oligonucleotide comprising a first segment having a nucleotide sequence that is substantially complementary to a segment of HBV nucleic acid and a second segment that is substantially complementary to an oligonucleotide bound to a solid phase;

(b) contacting the product of step (a) under hybridizing conditions with said oligonucleotide bound to the solid phase;

(c) thereafter separating materials not bound to the solid phase;

(d) contacting the bound product of step (c) under hybridization conditions with the nucleic acid multimer, said multimer comprising at least one oligonucleotide unit that is substantially complementary to the second segment of the amplifier probe polynucleotide and a multiplicity of second

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oligonucleotide units that are substantially complementary to a labeled oligonucleotide;

(e) removing unbound multimer;

(f) contacting under hybridizing conditions the

5 solid phase complex product of step (e) with the labeled oligonucleotide;

(g) removing unbound labeled oligonucleotide;

and

(h) detecting the presence of label in the

10 solid phase complex product of step (g).

Another aspect of the invention is a kit for the detection of HBV comprising a kit for the detection of HBV in a sample comprising in combination

(i) a set of amplifier probe oligonucleotides

15 wherein the amplifier probe oligonucleotide comprises a first segment having a nucleotide sequence substantially complementary to a segment of HBV nucleic acid and a second segment having a nucleotide sequence substantially complementary to an oligonucleotide unit of a nucleic  
20 acid multimer;

(ii) a set of capture probe oligonucleotides

wherein the capture probe oligonucleotide comprises a first segment having a nucleotide sequence that is substantially complementary to a segment of HBV nucleic  
25 acid and a second segment that is substantially complementary to an oligonucleotide bound to a solid phase;

(iii) a nucleic acid multimer, said multimer

30 comprising at least one oligonucleotide unit that is substantially complementary to the second segment of the amplifier probe polynucleotide and a multiplicity of second oligonucleotide units that are substantially complementary to a labeled oligonucleotide; and

(iv) a labeled oligonucleotide.

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These and other embodiments will readily occur to those of ordinary skill in view of the disclosure herein.

Modes for Carrying out the Invention

5 Definitions

In defining the present invention, the following terms will be employed, and are intended to be defined as indicated below.

10 "Solution phase nucleic acid hybridization assay" intends the assay techniques described and claimed in commonly owned U.S. Patent No. 4,868,105 and EPA 883096976.

15 A "modified nucleotide" intends a nucleotide monomer that may be stably incorporated into a polynucleotide and which has an additional functional group. Preferably, the modified nucleotide is a 5'-cytidine in which the N<sup>4</sup>-position is modified to provide a functional hydroxy group.

20 An "amplifier multimer" intends a branched polynucleotide that is capable of hybridizing simultaneously directly or indirectly to analyte nucleic acid and to a multiplicity of polynucleotide iterations (i.e., either iterations of another multimer or iterations of a labeled probe). The branching in the  
25 multimers is effected through covalent bonds and the multimers are composed of two types of oligonucleotide units that are capable of hybridizing, respectively, to analyte nucleic acid or nucleic acid hybridized to  
30 probes. The composition and preparation of such multimers are described in EPA 883096976 and U.S. Serial No. 558,897 filed 27 July 1990, the disclosures of which are incorporated herein by reference.

35 The term "amplifier probe" is intended as a branched or linear polynucleotide that is constructed to



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have a segment that hybridizes specifically to the analyte nucleic acid and iterations of a second segment that hybridize specifically to an amplifier multimer.

5 The term "capture probe" is intended as an oligonucleotide having a segment substantially complementary to a nucleotide sequence of the target DNA and a segment that is substantially complementary to a nucleotide sequence of a solid-phase-immobilized probe.

10 "Large" as used herein to describe the comb-type branched polynucleotides of the invention intends a molecule having at least about 15 branch sites and at least about 20 iterations of the labeled probe binding sequence.

15 "Comb-type" as used herein to describe the structure of the branched polynucleotides of the invention intends a polynucleotide having a linear backbone with a multiplicity of sidechains extending from the backbone.

20 A "cleavable linker molecule" intends a molecule that may be stably incorporated into a polynucleotide chain and which includes a covalent bond that may be broken or cleaved by chemical treatment or physical treatment such as by irradiation.

25 All nucleic acid sequences disclosed herein are written in a 5' to 3' direction unless otherwise indicated. Nucleotides are designated according to the nucleotide symbols recommended by the IUPAC-IUB Biochemical Nomenclature.

30 Solution Phase Hybridization Assay

The general protocol for the solution phase sandwich hybridizations is as follows. The analyte nucleic acid is placed in a microtiter well with an excess of two single-stranded nucleic acid probe sets:

35 (1) a set of capture probes, each having a first binding

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sequence substantially complementary to the analyte and a second binding sequence that is substantially complementary to nucleic acid bound to a solid support, for example, the well surface or a bead, and (2) a set of  
5 amplifier probes (branched or linear), each having a first binding sequence that is capable of specific binding to the analyte and a second binding sequence that is capable of specific binding to a segment of the multimer. The resulting product is a three component  
10 nucleic acid complex of the two probes hybridized to the analyte by their first binding sequences. The second binding sequences of the probes remain as single-stranded segments as they are not complementary to the analyte. This complex hybridizes to the immobilized probe on the  
15 solid surface via the second binding sequence of the capture probe. The resulting product comprises the complex bound to the solid surface via the duplex formed by the oligonucleotide bound to the solid surface and the second binding sequence of the capture probe. Unbound  
20 materials are then removed from the surface such as by washing.

The amplification multimer is then added to the bound complex under hybridization conditions to permit the multimer to hybridize to the available second binding  
25 sequence(s) of the amplifier probe of the complex. The resulting complex is then separated from any unbound multimer by washing. The labeled oligonucleotide is then added under conditions which permit it to hybridize to the substantially complementary oligonucleotide units of  
30 the multimer. The resulting immobilized labeled nucleic acid complex is then washed to remove unbound labeled oligonucleotide, and read.

The analyte nucleic acids may be from a variety of sources, e.g., biological fluids or solids, and may be  
35 prepared for the hybridization analysis by a variety of

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means, e.g., proteinase K/SDS, chaotropic salts, etc. Also, it may be of advantage to decrease the average size of the analyte nucleic acids by enzymatic, physical or chemical means, e.g., restriction enzymes, sonication, chemical degradation (e.g., metal ions), etc. The fragments may be as small as 0.1 kb, usually being at least about 0.5 kb and may be 1 kb or higher. The analyte sequence is provided in single-stranded form for analysis. Where the sequence is naturally present in single-stranded form, denaturation will not be required. However, where the sequence may be present in double-stranded form, the sequence should be denatured. Denaturation can be carried out by various techniques, such as alkali, generally from about 0.05 to 0.2 M hydroxide, formamide, salts, heat, enzymes, or combinations thereof.

The first binding sequences of the capture probe and amplifier probe that are substantially complementary to the analyte sequence will each be of at least 15 nucleotides, usually at least 25 nucleotides, and not more than about 5 kb, usually not more than about 1 kb, preferably not more than about 100 nucleotides. They will typically be approximately 30 nucleotides. They will normally be chosen to bind to different sequences of the analyte. The first binding sequences may be selected based on a variety of considerations. Depending upon the nature of the analyte, one may be interested in a consensus sequence, a sequence associated with polymorphisms, a particular phenotype or genotype, a particular strain, or the like.

The number of different amplifier and capture probes used influences the sensitivity of the assay, because the more probe sequences used, the greater the signal provided by the assay system. Furthermore, the use of more probe sequences allows the use of more

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stringent hybridization conditions, thereby reducing the incidence of false positive results. Thus, the number of probes in a set will be at least one capture probe and at least one amplifier probe, more preferably two capture  
5 and two amplifier probes, and most preferably 5-100 capture probes and 5-100 amplifier probes.

Probes for HBV were designed as follows.  
EPA 88309676 discloses a set of HBV probes designed by comparing the DNA sequences of the nine HBV subtypes  
10 reported in GenBank. Subsequent experimental analysis has demonstrated that these probes were complementary to the subgenomic strand (i.e, plus sense) of the incompletely double-stranded region of HBV, and thus different subsets of these probes hybridized to different  
15 viruses, since the length of the subgenomic strands varies among strains. Accordingly, the probe set has been redesigned to comprise sequences substantially complementary to the genomic-length strand (i.e, minus-sense) of HBV and to contain fewer spacer regions so as  
20 to include more oligonucleotides in the probe set, thereby increasing the sensitivity of the assay system.

In general, regions of greatest homology between the HBV isolates were selected as capture probes, while regions of lesser homology were selected as  
25 amplifier probes. Thus, as additional strains or isolates of HBV are made available, appropriate probes made be designed by aligning the sequence of the new strain or isolate with the nucleotide sequences used to design the probes of the present invention, and choosing  
30 regions of greatest homology for use as capture probes, with regions of lesser homology chosen as amplifier probes. The set of presently preferred probes and their capture or amplifier overhang regions, i.e., the regions which hybridize to sequences immobilized on solid support  
35 or to an amplifier multimer, are listed in the examples.

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The second binding sequences of the capture probe and amplifier probe are selected to be substantially complementary, respectively, to the oligonucleotide bound to the solid surface and to a  
5 segment of the multimer and so as to not be encountered by endogenous sequences in the sample/analyte. The second binding sequence may be contiguous to the first binding sequence or be spaced therefrom by an intermediate noncomplementary sequence. The probes may  
10 include other noncomplementary sequences if desired. These noncomplementary sequences must not hinder the binding of the binding sequences or cause nonspecific binding to occur.

The capture probe and amplifier probe may be  
15 prepared by oligonucleotide synthesis procedures or by cloning, preferably the former.

It will be appreciated that the binding sequences need not have perfect complementarity to provide homoduplexes. In many situations, heteroduplexes  
20 will suffice where fewer than about 10% of the bases are mismatches, ignoring loops of five or more nucleotides. Accordingly, as used herein the term "complementary" intends exact complementarity wherein each base within the binding region corresponds exactly, and  
25 "substantially complementary" intends 90% or greater homology.

The labeled oligonucleotide will include a sequence substantially complementary to the repeated oligonucleotide units of the multimer. The labeled  
30 oligonucleotide will include one or more molecules ("labels"), which directly or indirectly provide a detectable signal. The labels may be bound to individual members of the substantially complementary sequence or may be present as a terminal member or terminal tail  
35 having a plurality of labels. Various means for

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providing labels bound to the oligonucleotide sequences have been reported in the literature. See, for example, Leary et al., Proc. Natl. Acad. Sci. USA (1983) 80:4045; Renz and Kurz, Nucl. Acids Res. (1984) 12:3435; 5 Richardson and Gumpert, Nucl. Acids Res. (1983) 11:6167; Smith et al., Nucl. Acids Res. (1985) 13:2399; Meinkoth and Wahl, Anal. Biochem. (1984) 138:267. The labels may be bound either covalently or non-covalently to the substantially complementary sequence. Labels which may 10 be employed include radionuclides, fluorescers, chemilumescers, dyes, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, enzyme subunits, metal ions, and the like. Illustrative specific labels include fluorescein, rhodamine, Texas red, phycoerythrin, 15 umbelliferone, luminol, NADPH,  $\alpha$ - $\beta$ -galactosidase, horseradish peroxidase, alkaline phosphatase, etc.

The ratio of capture probe and amplifier probe to anticipated moles of analyte will each be at least stoichiometric and preferably in excess. This ratio is 20 preferably at least about 1.5:1, and more preferably at least 2:1. It will normally be in the range of 2:1 to  $10^6$ :1. Concentrations of each of the probes will generally range from about  $10^{-5}$  to  $10^{-9}$  M, with sample nucleic acid concentrations varying from  $10^{-21}$  to  $10^{-12}$  25 M. The hybridization steps of the assay will generally take from about 10 minutes to 20 hours, frequently being completed in about 1 hour. Hybridization can be carried out at a mildly elevated temperature, generally in the range from about 20°C to 80°C, more usually from about 30 35°C to 70°C, particularly 65°C.

The hybridization reactions are usually done in an aqueous medium, particularly a buffered aqueous medium, which may include various additives. Additives which may be employed include low concentrations of 35 detergent (0.01 to 1%), salts, e.g., sodium citrate

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(0.017 to 0.17 M), Ficoll, polyvinylpyrrolidone, carrier nucleic acids, carrier proteins, etc. Nonaqueous solvents may be added to the aqueous medium, such as dimethylformamide, dimethylsulfoxide, alcohols, and  
5 formamide. These other solvents are generally present in amounts ranging from 2 to 50%.

The stringency of the hybridization medium may be controlled by temperature, salt concentration, solvent system, and the like. Thus, depending upon the length  
10 and nature of the sequence of interest, the stringency will be varied.

Depending upon the nature of the label, various techniques can be employed for detecting the presence of the label. For fluorescers, a large number of different  
15 fluorometers are available. For chemiluminescers, luminometers or films are available. With enzymes, a fluorescent, chemiluminescent, or colored product can be provided and determined fluorometrically, luminometrically, spectrophotometrically or visually.  
20 The various labels which have been employed in immunoassays and the techniques applicable to immunoassays can be employed with the subject assays.

Kits for carrying out amplified nucleic acid hybridization assays according to the invention will  
25 comprise in packaged combination the following reagents: the amplifier probe or set of probes; the capture probe or set of probes; the amplifier multimer; and an appropriate labeled oligonucleotide. These reagents will typically be in separate containers in the kit. The kit  
30 may also include a denaturation reagent for denaturing the analyte, hybridization buffers, wash solutions, enzyme substrates, negative and positive controls and written instructions for carrying out the assay.

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The following examples further illustrate the invention. These examples are not intended to limit the invention in any manner.

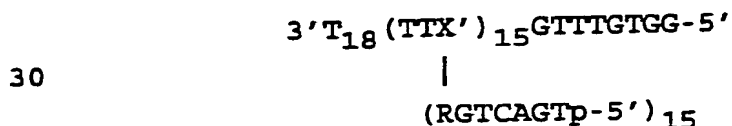
5

EXAMPLESExample ISynthesis of Comb-type Branched Polynucleotide

This example illustrates the synthesis of a comb-type branched polynucleotide having 15 branch sites and sidechain extensions having three labeled probe binding sites. This polynucleotide was designed to be used in a solution phase hybridization as described in EPA 883096976.

All chemical syntheses of oligonucleotides were performed on an automatic DNA synthesizer (Applied Biosystems, Inc., (ABI) model 380 B). Phosphoramidite chemistry of the beta cyanoethyl type was used including 5'-phosphorylation which employed Phostel™ reagent (ABN). Standard ABI protocols were used except as indicated. Where it is indicated that a multiple of a cycle was used (e.g., 1.2 cycle), the multiple of the standard amount of amidite recommended by ABI was employed in the specified cycle. Appended hereto are the programs for carrying out cycles 1.2 and 6.4 as run on the Applied Biosystems Model 380 B DNA Synthesizer.

A comb body of the following structure was first prepared:



wherein X' is a branching monomer, and R is a periodate cleavable linker.

The portion of the comb body through the 15 (TTX') repeats is first synthesized using 33.8 mg



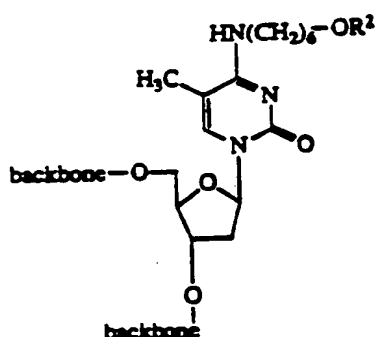
-15-

aminopropyl-derivatized thymidine controlled pore glass (CPG) (2000 Å, 7.4 micromoles thymidine per gram support) with a 1.2 cycle protocol. The branching site nucleotide was of the formula:

5

10

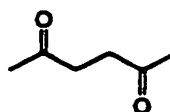
15



20

25

where R<sup>2</sup> represents



30

For synthesis of the comb body (not including sidechains), the concentration of beta  
35 cyanoethylphosphoramidite monomers was 0.1 M for A, C, G

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and T, 0.15 M for the branching site monomer E, and 0.2 M for Phostel™ reagent. Detritylation was done with 3% trichloroacetic acid in methylene chloride using stepped flowthrough for the duration of the deprotection. At the  
5 conclusion the 5' DMT was replaced with an acetyl group.

Cleavable linker R and six base sidechain extensions of the formula 3'-RGTCAGTp (SEQ ID NO:1) were synthesized at each branching monomer site as follows. The base protecting group removal ( $R^2$  in the formula  
10 above) was performed manually while retaining the CPG support in the same column used for synthesizing the comb body. In the case of  $R^2$  = levulinyl, a solution of 0.5 M hydrazine hydrate in pyridine/glacial acetic acid (1:1  
15 v/v) was introduced and kept in contact with the CPG support for 90 min with renewal of the liquid every 15 min, followed by extensive washing with pyridine/glacial acetic acid (1:1 v/v) and then by acetonitrile. After the deprotection the cleavable linker R and six base  
20 sidechain extensions were added using a 6.4 cycle.

In these syntheses the concentration of phosphoramidites was 0.1 M (except 0.2 M R and Phostel™ reagent; R was 2-(4-(4-(2-Dimethoxytrityloxy)ethyl)-phenoxy 2,3-di(benzoyloxy)-  
25 butyloxy)phenyl)ethyl-2-cyanoethyl-N,N-diisopropylphosphoramidite).

Detritylation is effected with a solution of 3% trichloroacetic acid in methylene chloride using continuous flowthrough, followed by a rinse solution of toluene/chloromethane (1:1 v/v). Branched  
30 polynucleotide chains were removed from the solid supports automatically in the 380B using the cycle "CE NH<sub>3</sub>." The ammonium hydroxide solution was collected in 4 ml screw-capped Wheaton vials and heated at 60°C for 12 hr to remove all base-protecting groups. After cooling  
35 to room temperature the solvent was removed in a Speed-

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Vac evaporator and the residue dissolved in 100  $\mu$ l water.

3' backbone extensions (segment A), sidechain extensions and ligation template/linkers of the following structures were also made using the automatic

5. synthesizer:

3' Backbone

extension 3'-TCCGTATCCTGGGCACAGAGGTGCP-5' (SEQ ID NO:2)

Sidechain

10 extension 3'-GATGCG(TTCATGCTGTTGGTGTAG)<sub>3</sub>-5' (SEQ ID NO:3)

Ligation

template for

linking 3'

backbone

extension 3'-AAAAAAAAAAGCACCTp-5' (SEQ ID NO:4)

15 Ligation tem-  
plate for link-  
ing sidechain

extension 3'-CGCATCACTGAC-5' (SEQ ID NO:5)

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The crude comb body was purified by a standard polyacrylamide gel (7% with 7 M urea and 1X TBE running buffer) method.

5 The 3' backbone extension and the sidechain extensions were ligated to the comb body as follows. The comb body (4 pmole/ $\mu$ l), 3' backbone extension (6.25 pmole/ $\mu$ l), sidechain extension (93.75 pmole/ $\mu$ l), sidechain linking template (75 pmoles/ $\mu$ l) and backbone linking template (5 pmole/ $\mu$ l) were combined in 1 mM ATP/  
10 5 mM DTT/ 50 mM Tris-HCl, pH 8.0/ 10 mM MgCl<sub>2</sub>/ 2 mM spermidine, with 0.5 units/ $\mu$ l T4 polynucleotide kinase. The mixture was incubated at 37°C for 2 hr, then heated in a water bath to 95°C, and then slowly cooled to below 35°C over a 1 hr period. 2 mM ATP, 10 mM DTT, 14%  
15 polyethylene glycol, and 0.21 units/ $\mu$ l T4 ligase were added, and the mixture incubated for 16-24 hr at 23°C. The DNA was precipitated in NaCl/ethanol, resuspended in water, and subjected to a second ligation as follows. The mixture was adjusted to 1 mM ATP, 5 mM DTT, 14%  
20 polyethylene glycol, 50 mM Tris-HCl, pH 7.5, 10 mM MgCl<sub>2</sub>, 2 mM spermidine, 0.5 units/ $\mu$ l T4 polynucleotide kinase, and 0.21 units/ $\mu$ l T4 ligase were added, and the mixture incubated at 23°C for 16-24 hr. Ligation products were then purified by polyacrylamide gel electrophoresis.

25 After ligation and purification, a portion of the product was labeled with <sup>32</sup>P and subjected to cleavage at the site of R achieved by oxidation with aqueous NaIO<sub>4</sub> for 1 hr. The sample was then analyzed by PAGE to determine the number of sidechain extensions  
30 incorporated by quantitating the radioactive label in the bands on the gel. The product was found to have a total of 45 labeled probe binding sites.

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EXAMPLE 2Hybridization Assay for HBV DNA

A "15 X 3" amplified solution phase nucleic acid sandwich hybridization assay format was employed in this example. The "15 x 3" designation derives from the fact that the format employs two multimers: (1) an amplifier probe having a first segment (A) that binds to HBV nucleic acid and a second segment (B) that hybridizes to (2) an amplifier multimer having a first segment (B\*) that hybridizes to the segment (B) and fifteen iterations of a segment (C), wherein segment C hybridizes to three labeled oligonucleotides.

The amplifier and capture probe segments and their respective names used in this assay were as follows.

HBV Amplifier Probes

HBV.104\* (SEQ ID NO:6)  
TTGTGGGTCTTTTGGGYTTTGCTGCTGCCWT  
HBV.94\* (SEQ ID NO:7)  
CCTKCTCGTGTTACAGGCGGGGTTTTCTT  
HBV.76\* (SEQ ID NO:8)  
TCCATGGCTGCTAGGSTGTRCTGCCAACTG  
HBV.87\* (SEQ ID NO:9)  
GCYTAYAGACCACCAAATGCCCCCTATCYTA  
HBV.45\* (SEQ ID NO:10)  
CTGTTCAAGCCTCCAAGCTGTGCCTTGGGT  
HBV.93\* (SEQ ID NO:11)  
CATGGAGARCAYMACATCAGGATTCCTAGG  
HBV.99\* (SEQ ID NO:12)  
TCCTGGYTATCGCTGGATGTGTCTGCGGCGT  
HBV.78\* (SEQ ID NO:13)  
GGCGCTGAATCCYGCGGACGACCCBTCTCG  
HBV.81\* (SEQ ID NO:14)  
CTTCGCTTCACCTCTGCACGTHGCATGGMG  
HBV.73\*070590-C (SEQ ID NO:15)

-20-

GGTCTSTGCCAAGTGTTTGCTGACGCAACC  
HBV.77\*070590-b (SEQ ID NO:16)  
CCTKCGCGGGACGTCCTTTGTYTACGTCCC  
HBV.D44\*070590-A (SEQ ID NO:17)  
5 MCCTCTGCCTAATCATCTCWTGTWCATGTC  
HBV.79\* (SEQ ID NO:18)  
CGACCACGGGGCGCACCTCTCTTTACGCGG  
HBV.82\* (SEQ ID NO:19)  
TGCCCAAGGTCTTACAYAAGAGGACTCTTG  
10 HBV.71\* (SEQ ID NO:20)  
CGTCAATCTYCKCGAGGACTGGGGACCCTG  
HBV.102\* (SEQ ID NO:21)  
ATGTTGCCCGTTTGTCCTCTAMTTCCAGGA  
HBV.101\* (SEQ ID NO:22)  
15 ATCTTCTTTRTTGGTTCTTCTGGAYTAYCAA  
HBV.100\* (SEQ ID NO:23)  
ATCATMTTCCTCTTCATCCTGCTGCTATGC  
HBV.98\* (SEQ ID NO:24)  
CAATCACTCACCAACCTCYTGTCTCCAAY  
20 HBV.97\* (SEQ ID NO:25)  
GTGTCYTGCCCAAATTCGCAGTCCCAAC  
HBV.96\* (SEQ ID NO:26)  
CTCGTGGTGGACTTCTCTCAATTTTCTAGG  
HBV.95\* (SEQ ID NO:27)  
25 GACAAGAATCCTCACAATACCRACAGAGTCT  
HBV.92\* (SEQ ID NO:28)  
TTTGGGGTGGAGCCCKCAGGCTCAGGGCR  
HBV.91\* (SEQ ID NO:29)  
CACCATATTCTTGGAACAAGAKCTACAGC  
30 HBV.88\* (SEQ ID NO:30)  
ACACTTCCGGARACTACTGTTGTTAGACGA  
HBV.86\* (SEQ ID NO:31)  
GTVTCTTTYGGAGTGTGGATTTCGCACTCCT  
HBV.D47\* (SEQ ID NO:32)  
35 TTGGAGCWWCTGTGGAGTTACTCTCKTTTT

-21-

HBV.D46\* (SEQ ID NO:33)  
TTTGGGGCATGGACATYGAYCCKTATAAAG  
HBV.85\* (SEQ ID NO:34)  
AAWGRTCTTTGTAYTAGGAGGCTGTAGGCA  
5 HBV.84\* (SEQ ID NO:35)  
RGA CTGGGAGGAGYTGGGGGAGGAGATTAG  
HBV.83\* (SEQ ID NO:36)  
CCTTGAGGCMTACTTCAAAGACTGTKTGTT  
HBV.80\* (SEQ ID NO:37)  
10 GTCTGTGCCCTTCTCATCTGCCGGWCCGTGT  
HBV.75\* (SEQ ID NO:38)  
AGCMGCTTGTTTTGCTCGCAGSMGGTCTGG  
HBV.74\* (SEQ ID NO:39)  
GGCTCSTCTGCCGATCCATACTGCGGAACT  
15 HBV.72\* (SEQ ID NO:40)  
MTKAACCTTTACCCCGTTGCTCGGCAACGG  
HBV.51\* (SEQ ID NO:41)  
GTGGCTCCAGTTCMGGAAACAGTAAACCCTG  
HBV.67\* (SEQ ID NO:42)  
20 KAARCAGGCTTTYACTTTCTCGCCAACTTA  
HBV.70\* 062890-A (SEQ ID NO:43)  
CCTCCKCCTGCCTCYACCAATCGSCAGTCA  
HBV.65\* (SEQ ID NO:44)  
ACCAATTTTCTTYTGTCTYTG GGTATACAT  
25

HBV Capture Probes

HBV.60\* (SEQ ID NO:45)  
TATTCCCATCCCATCTTCCTGGGCTTTCGS  
HBV.64\* (SEQ ID NO:46)  
30 TATATGGATGATGTGGTATTGGGGGCCAAG  
HBV.63\* (SEQ ID NO:47)  
CGTAGGGCTTTCCCCCACTGTTTGGCTTTC  
HBV.62\* (SEQ ID NO:48)  
GCTCAGTTTACTAGTGCCATTTGTTTCAGTG  
35 HBV.61\* (SEQ ID NO:49)

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CCTATGGGAGKGGGCCTCAGYCCGTTTCTC  
HBV.89\* (SEQ ID NO:50)

GTCCCTAGAAGAAGAACTCCCTCGCCTCG  
HBV.90\* (SEQ ID NO:51)

5 ACGMAGRTCTCMATCGCCGCGTCGCAGAAGA  
HBV.D13\* (SEQ ID NO:52)

CAATCTCGGGAATCTCAATGTTAGTATYCC  
HBV.D14\* (SEQ ID NO:53)

GACTCATAAGGTSGGRAACTTTACKGGGCT  
10

Each amplifier probe contained, in addition to the sequences substantially complementary to the HBV sequences, the following 5' extension complementary to a segment of the amplifier multimer,

15 AGGCATAGGACCCGTGTCTT (SEQ ID NO:54).

Each capture probe contained, in addition to the sequences substantially complementary to HBV DNA, the following downstream sequence complementary to DNA bound to the solid phase (i.e., complementary to XT1\*),

20 CTTCCTTGGAGAAAGTGGTG (SEQ ID NO:55).

Microtiter plates were prepared as follows. White Microlite 1 Removawell strips (polystyrene microtiter plates, 96 wells/plate) were purchased from Dynatech Inc. Each well was filled with 200  $\mu$ l 1 N HCl and incubated at room temperature for 15-20 min. The plates were then washed 4 times with 1X PBS and the wells aspirated to remove liquid. The wells were then filled with 200  $\mu$ l 1 N NaOH and incubated at room temperature for 15-20 min. The plates were again washed 4 times with 1X PBS and the wells aspirated to remove liquid.

30 Poly(phe-lys) was purchased from Sigma Chemicals, Inc. This polypeptide has a 1:1 molar ratio of phe:lys and an average m.w. of 47,900 gm/mole. It has an averag length of 309 amino acids and contains 155



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amines/mole. A 1 mg/ml solution of the polypeptide was mixed with 2M NaCl/1X PBS to a final concentration of 0.1 mg/ml (pH 6.0). 100  $\mu$ l of this solution was added to each well. The plate was wrapped in plastic to prevent drying and incubated at 30°C overnight. The plate was then washed 4 times with 1X PBS and the wells aspirated to remove liquid.

The following procedure was used to couple the oligonucleotide XT1\* to the plates. Synthesis of XT1\* was described in EPA 883096976. 20 mg disuccinimidyl suberate was dissolved in 300  $\mu$ l dimethyl formamide (DMF). 26 OD<sub>260</sub> units of XT1\* was added to 100  $\mu$ l coupling buffer (50 mM sodium phosphate, pH 7.8). The coupling mixture was then added to the DSS-DMF solution and stirred with a magnetic stirrer for 30 min. An NAP-25 column was equilibrated with 10 mM sodium phosphate, pH 6.5. The coupling mixture DSS-DMF solution was added to 2 ml 10 mM sodium phosphate, pH 6.5, at 4°C. The mixture was vortexed to mix and loaded onto the equilibrated NAP-25 column. DSS-activated XT1\* DNA was eluted from the column with 3.5 ml 10 mM sodium phosphate, pH 6.5. 5.6 OD<sub>260</sub> units of eluted DSS-activated XT1\* DNA was added to 1500 ml 50 mM sodium phosphate, pH 7.8. 50  $\mu$ l of this solution was added to each well and the plates were incubated overnight. The plate was then washed 4 times with 1X PBS and the wells aspirated to remove liquid.

Final stripping of plates was accomplished as follows. 200  $\mu$ L of 0.2N NaOH containing 0.5% (w/v) SDS was added to each well. The plate was wrapped in plastic and incubated at 65°C for 60 min. The plate was then washed 4 times with 1X PBS and the wells aspirated to remove liquid. The stripped plate was stored with desiccant beads at 2-8°C.

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Sample preparation consisted of delivering 12.5  $\mu$ l P-K buffer (2 mg/ml proteinase K in 10 mM Tris-HCl, pH 8.0/ 0.15 M NaCl/ 10 mM EDTA, pH 8.0/ 1% SDS/ 40  $\mu$ g/ml sonicated salmon sperm DNA) to each well.

- 5 A standard curve of HBV DNA was prepared by diluting cloned HBV, subtype adw, DNA in HBV negative human serum and delivering aliquots of dilutions corresponding to 1000, 3000, 10,000, 30,000, or 100,000 molecules to each well. Tests for cross-hybridization to heterologous DNAs  
10 were done by adding either purified DNA or infected cells to each well. Amounts for each organism are indicated in the Table.

- Plates were covered and agitated to mix samples, then incubated at 65° C to release nucleic  
15 acids.

- A cocktail of the HBV-specific amplifier and capture probes listed above was added to each well (5 fmoles of each probe/well, diluted in 1 N NaOH). Plates were covered and gently agitated to mix reagents and then  
20 incubated at 65° C for 30 min.

Neutralization buffer was then added to each well (0.77 M 3-(N-morpholino)propane sulfonic acid/1.845 M NaCl/0.185 sodium citrate). Plates were covered and incubated for 12-18 hr at 65° C.

- 25 After an additional 10 min at room temperature, the contents of each well were aspirated to remove all fluid, and the wells washed 2X with washing buffer (0.1% SDS/0.015 M NaCl/ 0.0015 sodium citrate).

- Amplifier multimer was then added to each well  
30 (30 fmoles/well). After covering plates and agitating to mix the contents in the wells, the plates were incubated for 30 min at 55° C.

After a further 5-10 min period at room temperature, the wells were washed as described above.

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Alkaline phosphatase label probe, disclosed in EP 883096976, was then added to each well (40  $\mu$ l/well of 2.5 fmoles/ $\mu$ l). After incubation at 55°C for 15 min, and 5 min at room temperature, the wells were washed twice as above and then 3X with 0.015 M NaCl/0.0015 M sodium citrate.

An enzyme-triggered dioxetane (Schaap et al., Tet. Lett. (1987) 28:1159-1162 and EPA Pub. No. 0254051), obtained from Lumigen, Inc., was employed. 20  $\mu$ l Lumiphos 530 (Lumigen) was added to each well. The wells were tapped lightly so that the reagent would fall to the bottom and gently swirled to distribute the reagent evenly over the bottom. The wells were covered and incubated at 37°C for 40 min.

Plates were then read on a Dynatech ML 1000 luminometer. Output was given as the full integral of the light produced during the reaction.

Results from an exclusivity study of the HBV probes is shown in the Table below. Results for each standard sample are expressed as the difference between the mean of the negative control plus two standard deviations and the mean of the sample minus two standard deviations (delta). If delta is greater than zero, the sample is considered positive. These results indicate the ability of these probe sets to distinguish HBV DNA from heterologous organisms and a sensitivity of about 1000-3000 HBV molecules.

Table		
Sample	Amount	Delta
HBV	1 X 10 <sup>5</sup>	25.99
HBV	3 X 10 <sup>4</sup>	6.51
HBV	1 X 10 <sup>4</sup>	3.00
HBV	3 X 10 <sup>3</sup>	0.93
HBV	1 X 10 <sup>3</sup>	-0.20

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	Control	--	--
	HCV	8 X 10 <sup>5</sup>	-0.39
	CMV <sup>1</sup>	3.3 X 10 <sup>6</sup>	-0.48
	HTLV-II <sup>2</sup>	1 X 10 <sup>5</sup>	-0.07
5	HTLV-I <sup>2</sup>	1 X 10 <sup>5</sup>	-0.23
	HIV	1 X 10 <sup>7</sup>	-0.31
	pBR325	1 X 10 <sup>7</sup>	-0.27
	Streptococcus sanguis	1 X 10 <sup>7</sup>	-0.31
	Streptococcus pyogenes	1 X 10 <sup>7</sup>	-0.36
10	Streptococcus pneumoniae	1 X 10 <sup>7</sup>	-0.38
	Streptococcus fecalis	1 X 10 <sup>7</sup>	-0.28
	Streptococcus agalactiae	1 X 10 <sup>7</sup>	-0.26
	Streptococcus epidermidis	1 X 10 <sup>7</sup>	-0.31
	Staphylococcus aureus	1 X 10 <sup>7</sup>	-0.34
15	Serratia marcescens	1 X 10 <sup>7</sup>	-0.30
	Pseudomonas aeruginosa	1 X 10 <sup>7</sup>	-0.23
	Proteus mirabilis	1 X 10 <sup>7</sup>	-0.43
	Peptostreptococcus	1 X 10 <sup>7</sup>	-0.46
	anerobius		
20	Lactobacillus acidophilus	1 X 10 <sup>7</sup>	-0.33
	Klebsiella pneumoniae	1 X 10 <sup>7</sup>	-0.12
	Haemophilus influenza	1 X 10 <sup>7</sup>	-0.34
	Escherichia coli	1 X 10 <sup>7</sup>	-0.44
	Enterobacter aerogenes	1 X 10 <sup>7</sup>	-0.23
25	Mycobacterium leprae	1 X 10 <sup>7</sup>	-0.18

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<sup>1</sup> denotes pfu in infected cells

<sup>2</sup> denotes proviral copies

30                    Modifications of the above-described modes for carrying out the invention that are obvious to those of skill in biochemistry, nucleic acid hybridization assays, and related fields are intended to be within the scope of the following claims.

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## SEQUENCE LISTING

## 5

(i) APPLICANT: Irvine, Bruce D.  
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Urdea, Michael S.

(11) TITLE OF INVENTION: HBV PROBES FOR USE IN SOLUTION  
PHASE SANDWICH HYBRIDIZATION ASSAYS

10 (iii) NUMBER OF SEQUENCES: 55

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15

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(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

20

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: 07/813,586  
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(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

25

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30

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

35

(A) LENGTH: 7 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGACTGR

7.

5 (2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 24 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CGTGGAGACA CGGGTCCTAT GCCT

24

15 (2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 60 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GATGTGGTTG TCGTACTTGA TGTGGTTGTC GTACTTGATG TGGTTGTCGT ACTTGCCTAG

60

25 (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 16 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TCCACGAAAA AAAAAA

16

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 12 base pairs

35

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- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CAGTCACTAC GC

12

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

15 TTGTGGGTCT TTGGGYTTT GCTGCTCCWT

30

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

25 CCTKCTCGTG TTACAGGCGG GGTTTTTCTT

30

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TCCATGGCTG CTAGGSTGTR CTGCCAACTG

30

35 (2) INFORMATION FOR SEQ ID NO:9:

-30-

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 30 base pairs  
    (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

5

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GCYTAYAGAC CACCAAATGC CCCTATCYTA

30

- (2) INFORMATION FOR SEQ ID NO:10:

10

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 30 base pairs  
    (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

15

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CTGTTCAAGC CTCCAAGCTG TGCCTTGGGT

30

- (2) INFORMATION FOR SEQ ID NO:11:

20

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 30 base pairs  
    (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

25

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CATGGAGARC AYMATCAG GATTCCTAGG

30

- (2) INFORMATION FOR SEQ ID NO:12:

30

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 31 base pairs  
    (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

35 TCCTGGYTAT CGCTGGATGT GTCGCGGCG T

31



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## (2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

5

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GGCGCTGAAT CCYCGGACG ACCCBTCTCG

30

## 10 (2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

15

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CTTCGCTTCA CCTCTGCACG THGCATGGMG

30

## (2) INFORMATION FOR SEQ ID NO:15:

20

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

25

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGTCTSTGCC AAGTGTTCG TGACGCAACC

30

## (2) INFORMATION FOR SEQ ID NO:16:

30

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

35

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CCTKCGCGGG ACGTCCTTTG TYTACGTCCC

30

(2) INFORMATION FOR SEQ ID NO:17:

- 5 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

MCCTCTGCCT AATCATCTCW TGTWCATGTC

30

(2) INFORMATION FOR SEQ ID NO:18:

- 15 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CGACCACGGG GCGCACCTCT CTTACGCGG

30

(2) INFORMATION FOR SEQ ID NO:19:

- 25 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

30 TGCCCAAGGT CTTACAYAAG AGGACTCTTG

30

(2) INFORMATION FOR SEQ ID NO:20:

- 35 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CGTCAATCTY CKCGAGGACT GGGGACCCTG

30

5 (2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

ATGTTGCCCG TTGTCCTCT AMTCCAGGA

30

(2) INFORMATION FOR SEQ ID NO:22:

15

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

ATCTTCTTCT TGGTCTTCT GGATTAYCAA

30

(2) INFORMATION FOR SEQ ID NO:23:

25

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

ATCATMTTCC TCTTCATCCT GCTGCTATGC

30

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

35

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(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

5

CAATCACTCA CCAACCTCYT GTCCTCCAAY

30

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

10

(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

15

GTGTCYTGGC CAAAATTGCG AGTCCCCAAC

30

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

20

(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CTCGTGGTGG ACTTCTCTCA ATTTCTAGG

30

25

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

30

(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GACAAGAATC CTCACAATAC CRCAGAGTCT

30

(2) INFORMATION FOR SEQ ID NO:28:

35

-35-

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

TTTTGGGGTG GAGCCCKCAG GCTCAGGGCR

30

(2) INFORMATION FOR SEQ ID NO:29:

10

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CACCATATTC TTGGGAACAA GAKCTACAGC

30

(2) INFORMATION FOR SEQ ID NO:30:

20

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

ACACTTCCGG ARACTACTGT TGTTAGACGA

30

(2) INFORMATION FOR SEQ ID NO:31:

30

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

35 GTVTCITTTYG GAGTGTGGAT TCGCACTCCT

30

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## (2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

5

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TTGGAGCWWC TGTGGAGTTA CTCTCKTTTT

30

## 10 (2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

15

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

TTTGGGGCAT GGACATYGAY CCKTATAAAG

30

## 20 (2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

25

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

AAWGRTCTTT GTAYTAGGAG GCTGTAGGCA

30

## (2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

30

35

-37-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

RGACTGGGAG GAGYTGGGGG AGGAGATTAG

30

(2) INFORMATION FOR SEQ ID NO:36:

- 5           (i) SEQUENCE CHARACTERISTICS:  
            (A) LENGTH: 30 base pairs  
            (B) TYPE: nucleic acid  
            (C) STRANDEDNESS: single  
            (D) TOPOLOGY: linear

10           (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

CCTTGAGGCM TACTTCAAAG ACTGKTGT

30

(2) INFORMATION FOR SEQ ID NO:37:

- 15           (i) SEQUENCE CHARACTERISTICS:  
            (A) LENGTH: 30 base pairs  
            (B) TYPE: nucleic acid  
            (C) STRANDEDNESS: single  
            (D) TOPOLOGY: linear

20           (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GTCTGTGCCT TCTCATCTGC CGGWCCGTGT

30

(2) INFORMATION FOR SEQ ID NO:38:

- 25           (i) SEQUENCE CHARACTERISTICS:  
            (A) LENGTH: 30 base pairs  
            (B) TYPE: nucleic acid  
            (C) STRANDEDNESS: single  
            (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

30           AGCMGCTTGT TTGCTCGCA GSMGGTCTGG

30

(2) INFORMATION FOR SEQ ID NO:39:

- 35           (i) SEQUENCE CHARACTERISTICS:  
            (A) LENGTH: 30 base pairs  
            (B) TYPE: nucleic acid  
            (C) STRANDEDNESS: single  
            (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GGCTCSICTG CCGATCCATA CTGCGGAAC

30

5 (2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

MTKAACCTTT ACCCCGTTGC TCGGCAACGG

30

(2) INFORMATION FOR SEQ ID NO:41:

- 15 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

GTGGCTCCAG TTCMGAACA GTAAACCCTG

30

(2) INFORMATION FOR SEQ ID NO:42:

- 25 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

KAARCAGGCT TTYACTTTCT CGCCAACTTA

30

(2) INFORMATION FOR SEQ ID NO:43:

- 35 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid



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- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:43: 30  
CCTCCKCCTG CCTCYACCAA TCGSCAGTCA

(2) INFORMATION FOR SEQ ID NO:44:

10 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44: 30  
15 ACCAATTTTC TTYTGTCTYT GGGTATACAT

(2) INFORMATION FOR SEQ ID NO:45:

20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45: 30  
TATTCCTATC CCATCCTCCT GGGCTTTTCS

25 (2) INFORMATION FOR SEQ ID NO:46:

30 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46: 30  
TATATGGATG ATGTGGTATT GGGGGCCAAG

35 (2) INFORMATION FOR SEQ ID NO:47:

-40-

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

CGTAGGGCTT TCCCCACTG TTTGGCTTTC

30

(2) INFORMATION FOR SEQ ID NO:48:

10

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

GCTCAGTTTA CTAGTGCCAT TTGTTCACTG

30

(2) INFORMATION FOR SEQ ID NO:49:

20

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

CCTATGGGAG KGGGCCTCAG YCCGTTTCTC

30

(2) INFORMATION FOR SEQ ID NO:50:

30

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

GTCCCCTAGA AGAAGAACTC CCTCGCCTCG

30

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## (2) INFORMATION FOR SEQ ID NO:51:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 31 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

5

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

ACGMAGRTCT CMATCGCCGC GTCGCAGAAG A

31

## 10 (2) INFORMATION FOR SEQ ID NO:52:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

15

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

CAATCTCGGG AATCTCAATG TTAGTATYCC

30

## 20 (2) INFORMATION FOR SEQ ID NO:53:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

25

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

GATCATAAG GTSGGAACT TTACKGGGCT

30

## (2) INFORMATION FOR SEQ ID NO:54:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

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35

-42-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

20

AGGCATAGGA CCCGTGTCTT

(2) INFORMATION FOR SEQ ID NO:55:

- 5 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

20

CTTCATTGGA GAAAGTGGTG

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43

FILE NAME	LAST ACCESS	DATE CREATED	FILE NAME	LAST ACCESS	DATE CREATED
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## FILE TYPE: SYNTHESIS CYCLE

6.4XSC-5	08	27, 1991	08	27, 1991	6.4XS-5	08	27, 1991	08	27, 1991
1.2XD-6	08	27, 1991	08	27, 1991	1.2X-6	08	27, 1991	08	27, 1991
ssceaf3	01	07, 1990	01	07, 1990	ceaf3	01	07, 1990	01	07, 1990
10ceaf3	01	07, 1990	01	07, 1990	hpaf3	01	07, 1990	01	07, 1990
10hpaf3	01	07, 1990	01	07, 1990	rnaaf3	01	07, 1990	01	07, 1990
10rnaaf3	01	07, 1990	01	07, 1990	ssceaf3	01	07, 1990	01	07, 1990
cef3	01	07, 1990	01	07, 1990	10ceaf3	01	07, 1990	01	07, 1990
10hpaf3	01	07, 1990	01	07, 1990	rnaaf3	01	07, 1990	01	07, 1990
10rnaaf3	01	07, 1990	01	07, 1990	ssceaf1	01	07, 1990	01	07, 1990
ceaf1	01	07, 1990	01	07, 1990	10ceaf1	01	07, 1990	01	07, 1990
hpaf1	01	07, 1990	01	07, 1990	10hpaf1	01	07, 1990	01	07, 1990
rnaaf1	01	07, 1990	01	07, 1990	10rnaaf1	01	07, 1990	01	07, 1990
ssceaf1	01	07, 1990	01	07, 1990	cef1	01	07, 1990	01	07, 1990
10ceaf1	01	07, 1990	01	07, 1990	10hpaf1	01	07, 1990	01	07, 1990
rnaaf1	01	07, 1990	01	07, 1990	10rnaaf1	01	07, 1990	01	07, 1990

## FILE TYPE: BOTTLE CHANGE PROCEDURE

bc 18	07	01, 1986	07	01, 1986	bc 17	07	01, 1986	07	01, 1986
bc 16	07	01, 1986	07	01, 1986	bc 15	07	01, 1986	07	01, 1986
bc 14	07	01, 1986	07	01, 1986	bc 13	07	01, 1986	07	01, 1986
bc 12	07	01, 1986	07	01, 1986	bc 11	07	01, 1986	07	01, 1986
bc 10	07	01, 1986	07	01, 1986	bc 9	07	01, 1986	07	01, 1986
bc 8a	07	01, 1986	07	01, 1986	bc 7	07	01, 1986	07	01, 1986
bc 6	07	01, 1986	07	01, 1986	bc 5	07	01, 1986	07	01, 1986
bc 4	07	01, 1986	07	01, 1986	bc 3	07	01, 1986	07	01, 1986
bc 2	07	01, 1986	07	01, 1986	bc 1	07	01, 1986	07	01, 1986

## FILE TYPE: END PROCEDURE

CAP-PRIM	08	27, 1991	08	27, 1991	CE NH3	08	27, 1991	08	27, 1991
deprce	10	08, 1990	10	08, 1990	deprce10	10	08, 1990	10	08, 1990
deprho	10	08, 1990	10	08, 1990	deprho10	10	08, 1990	10	08, 1990
deprna	10	08, 1990	10	08, 1990	deprna10	10	08, 1990	10	08, 1990

## FILE TYPE: BEGIN PROCEDURE

STD PREP	08	27, 1991	08	27, 1991	phos003	07	01, 1986	07	01, 1986
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## FILE TYPE: SHUT-DOWN PROCEDURE

clean003	07	01, 1986	07	01, 1986
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## FILE TYPE: DNA SEQUENCES

15X-2	08	27, 1991	08	27, 1991	15X-1	08	27, 1991	08	27, 1991
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44

STEP NUMBER	FUNCTION # NAME	STEP TIME	STEP ACTIVE FOR BASES							SAFE STEP
			A	G	C	T	S	S	7	
1	10 #18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9 #18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1 Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	5 Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	28 Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	+45 Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	19 B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	-46 Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	+47 Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	20 B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	-48 Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	+49 Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	21 B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	-50 Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	4 Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	+45 Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	19 B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	-46 Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	+47 Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	20 B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	-48 Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	+49 Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	21 B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	-50 Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	4 Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
39	+45 Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	19 B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
42	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
43	-46 Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

(C ntinued next page.)

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STEP NUMBER	FUNCTION # NAME	STEP TIME	STEP ACTIVE FOR BASES							SAFE STEP
			A	E	C	T	E	S	7	
44	+47 Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
45	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
46	20 B+TET To Col 2	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
47	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
48	-48 Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
49	+49 Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
50	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
51	21 B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
52	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
53	-50 Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
54	4 Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
55	+45 Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
56	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
57	19 B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
58	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
59	-46 Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
60	+47 Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
61	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
62	20 B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
63	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
64	-48 Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
65	+49 Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
66	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
67	21 B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
68	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
69	-50 Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
70	4 Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
71	+45 Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
72	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
73	19 B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
74	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
75	-46 Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
76	+47 Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
77	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
78	20 B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
79	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
80	-48 Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
81	+49 Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
82	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
83	21 B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
84	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
85	-50 Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
86	4 Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
87	+45 Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
88	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

(C ntinued next page.)

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STEP NUMBER	FUNCTION ± NAME	STEP TIME	STEP ACTIVE FOR BASES							SAFE STEP
			A	S	C	T	S	S	T	
99	19 B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
90	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
91	-46 Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
92	+47 Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
93	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
94	20 B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
95	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
96	-48 Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
97	+49 Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
98	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
99	21 B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
100	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
101	-50 Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
102	4 Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
103	+45 Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
104	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
105	19 B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
106	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
107	-46 Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
108	+47 Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
109	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
110	20 B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
111	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
112	-48 Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
113	+49 Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
114	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
115	21 B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
116	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
117	-50 Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
118	4 Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
119	+45 Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
120	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
121	19 B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
122	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
123	-46 Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
124	+47 Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
125	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
126	20 B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
127	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
128	-48 Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
129	+49 Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
130	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
131	21 B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
132	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
133	-50 Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

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STEP NUMBER	FUNCTION # NAME	STEP TIME	STEP ACTIVE FOR BASES							SAFE STEP
			A	G	C	T	S	S	7	
134	4 Wait	-30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
135	10 \$18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
136	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
137	1 Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
138	31 \$15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
139	13 \$15 To Column	22	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
140	10 \$18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
141	4 Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
142	2 Reverse Flush	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
143	1 Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
144	9 \$18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
145	34 Flush to Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
146	9 \$18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
147	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
148	9 \$18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
149	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
150	9 \$18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
151	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
152	1 Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
153	33 Cycle Entry	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
154	5 Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
155	37 Relay 3 Pulse	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
156	82 \$14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
157	30 \$17 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
158	10 \$18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
159	9 \$18 To Column	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
160	11 \$17 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	N
161	14 \$14 To Column	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
162	2 Reverse Flush	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
163	11 \$17 To Column	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	N
164	34 Flush to Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
165	11 \$17 To Column	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
166	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
167	14 \$14 To Column	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	N
168	34 Flush to Waste	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
169	7 Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
170	9 \$18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
171	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
172	9 \$18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
173	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
174	9 \$18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
175	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
176	1 Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

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STEP NUMBER	FUNCTION # NAME	STEP TIME	STEP ACTIVE FOR BASES							SAFE STEP
			A	S	C	T	S	S	7	
1	10 #18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9 #18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1 Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	5 Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	28 Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	+45 Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	19 B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	-46 Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	+47 Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	20 B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	-48 Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	+49 Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	21 B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	-50 Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	4 Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	+45 Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	19 B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	-46 Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	+47 Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	20 B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	-48 Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	+49 Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	21 B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	-50 Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	4 Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
39	+45 Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	19 B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
42	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
43	-46 Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

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STEP NUMBER	FUNCTION # NAME	STEP TIME	STEP ACTIVE FOR BASES							SAFE STEP
			A	G	C	T	E	S	7	
44	+47 Group 2 On	- 1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
45	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
46	20 B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
47	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
48	-48 Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
49	+49 Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
50	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
51	21 B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
52	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
53	-50 Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
54	4 Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
55	+45 Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
56	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
57	19 B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
58	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
59	-46 Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
60	+47 Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
61	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
62	20 B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
63	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
64	-48 Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
65	+49 Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
66	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
67	21 B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
68	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
69	-50 Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
70	4 Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
71	+45 Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
72	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
73	19 B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
74	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
75	-46 Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
76	+47 Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
77	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
78	20 B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
79	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
80	-48 Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
81	+49 Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
82	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
83	21 B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
84	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
85	-50 Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
86	4 Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
87	+45 Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
88	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

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STEP NUMBER	FUNCTION # NAME	STEP TIME	STEP ACTIVE FOR BASES							SAFE STEP
			A	G	C	T	5	5	7	
89	19 B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
90	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
91	-46 Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
92	+47 Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
93	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
94	20 B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
95	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
96	-48 Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
97	+49 Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
98	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
99	21 B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
99	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
100	-50 Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
101	4 Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
102	+45 Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
103	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
104	19 B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
105	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
106	-46 Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
107	+47 Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
108	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
109	20 B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
110	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
111	-48 Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
112	+49 Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
113	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
114	21 B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
115	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
116	-50 Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
117	4 Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
118	+45 Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
119	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
120	19 B+TET To Col 1	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
121	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
122	-46 Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
123	+47 Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
124	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
125	20 B+TET To Col 2	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
126	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
127	-48 Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
128	+49 Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
129	90 TET To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
130	21 B+TET To Col 3	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
131	90 TET To Column	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
132	-50 Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
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STEP NUMBER*	FUNCTION # NAME	STEP TIME	STEP ACTIVE FOR BASES							SAFE STEP
			A	G	C	T	S	S	7	
134	4 Wait	50	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
135	16 Cap Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
136	10 #18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
137	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
138	1 Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
139	91 Cap To Column	22	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
140	10 #18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
141	4 Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
142	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
143	1 Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
144	81 #15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
145	13 #15 To Column	22	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
146	10 #18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
147	4 Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
148	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
149	1 Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
150	9 #18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
151	34 Flush to Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
152	9 #18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
153	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
154	9 #18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
155	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
156	9 #18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
157	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
158	1 Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
159	33 Cycle Entry	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
160	6 Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
161	37 Relay 3 Pulse	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
162	82 #14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
163	30 #17 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
164	10 #18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
165	9 #18 To Column	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	N
166	11 #17 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
167	14 #14 To Column	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
168	2 Reverse Flush	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
169	11 #17 To Column	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
170	34 Flush to Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
171	11 #17 To Column	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
172	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
173	14 #14 To Column	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
174	34 Flush to Waste	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
175	7 Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
176	9 #18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
177	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
178	9 #18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

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STEP NUMBER	FUNCTION = NAME	STEP TIME	STEP ACTIVE FOR BASES								SAFE STEP
			A	G	C	T	S	S	7		
179	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	
180	3 218 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	
191	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	
182	1 Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	

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STEP NUMBER	FUNCTION # NAME	STEP TIME	STEP ACTIVE FOR BASES							SAFE STEP
			A	G	C	T	S	S	7	
1	10 \$18 To Waste	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9 \$18 To Column	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1 Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	5 Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	28 Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	+45 Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	90 TET To Column	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	19 B+TET To Col 1	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	90 TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	19 B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	90 TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	19 B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	9 \$18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	-46 Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	+47 Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	10 \$18 To Waste	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	1 Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	90 TET To Column	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	20 B+TET To Col 2	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	90 TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	20 B+TET To Col 2	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	90 TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	20 B+TET To Col 2	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	9 \$18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	-48 Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	+49 Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	10 \$18 To Waste	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	1 Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	90 TET To Column	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	21 B+TET To Col 3	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	90 TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	21 B+TET To Col 3	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	90 TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	21 B+TET To Col 3	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	9 \$18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	-50 Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	4 Wait	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
39	2 Reverse Flush	5						Yes		Yes
40	10 \$18 To Waste	2						Yes		Yes
41	9 \$18 To Column	9						Yes		Yes
42	2 Reverse Flush	5						Yes		Yes
43	10 \$18 T Waste	3						Yes		Yes

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STEP NUMBER	FUNCTION # NAME	STEP TIME	STEP ACTIVE FOR BASES							SAFE STEP
			A	G	C	T	E	6	7	
44	1 Block Flush	3						Yes		Yes
45	+45 Group 1 On	1						Yes		Yes
46	90 TET To Column	6						Yes		Yes
47	19 B+TET To Col 1	6						Yes		Yes
48	90 TET To Column	3						Yes		Yes
49	19 B+TET To Col 1	3						Yes		Yes
50	90 TET To Column	3						Yes		Yes
51	19 B+TET To Col 1	3						Yes		Yes
52	9 #18 To Column	1						Yes		Yes
53	-46 Group 1 Off	1						Yes		Yes
54	+47 Group 2 On	1						Yes		Yes
55	10 #18 To Waste	4						Yes		Yes
56	1 Block Flush	3						Yes		Yes
57	90 TET To Column	6						Yes		Yes
58	20 B+TET To Col 2	6						Yes		Yes
59	90 TET To Column	3						Yes		Yes
60	20 B+TET To Col 2	3						Yes		Yes
61	90 TET To Column	3						Yes		Yes
62	20 B+TET To Col 2	3						Yes		Yes
63	9 #18 To Column	1						Yes		Yes
64	-48 Group 2 Off	1						Yes		Yes
65	+49 Group 3 On	1						Yes		Yes
66	10 #18 To Waste	4						Yes		Yes
67	1 Block Flush	3						Yes		Yes
68	90 TET To Column	6						Yes		Yes
69	21 B+TET To Col 3	6						Yes		Yes
70	90 TET To Column	3						Yes		Yes
71	21 B+TET To Col 3	3						Yes		Yes
72	90 TET To Column	3						Yes		Yes
73	21 B+TET To Col 3	3						Yes		Yes
74	9 #18 To Column	1						Yes		Yes
75	-50 Group 3 Off	1						Yes		Yes
76	4 Wait	20						Yes		Yes
77	16 Cap Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
78	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
79	1 Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
80	91 Cap To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
81	10 #18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
82	4 Wait	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
83	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
84	81 #15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
85	13 #15 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
86	10 #18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
87	4 Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
88	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

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STEP NUMBER	FUNCTION # NAME	STEP TIME	STEP ACTIVE FOR BASES							SAFE STEP
			A	G	C	T	S	6	7	
1	10 \$18 To Waste	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9 \$18 To Column	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1 Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	5 Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	28 Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	+45 Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	90 TET To Column	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	19 B+TET To Col 1	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	90 TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	19 B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	90 TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	19 B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	9 \$18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	-46 Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	+47 Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	10 \$18 To Waste	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	1 Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	90 TET To Column	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	20 B+TET To Col 2	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	90 TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	20 B+TET To Col 2	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	90 TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	20 B+TET To Col 2	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	9 \$18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	-48 Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	+49 Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	10 \$18 To Waste	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	1 Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	90 TET To Column	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	21 B+TET To Col 3	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	90 TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	21 B+TET To Col 3	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	90 TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	21 B+TET To Col 3	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	9 \$18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	-50 Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	4 Wait	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
39	16 Cap Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	1 Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
42	91 Cap To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
43	10 \$18 T Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

(Continued next page.)

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STEP NUMBER	FUNCTION # NAME	STEP TIME	STEP ACTIVE FOR BASES							SAFE STEP
			A	S	C	T	5	6	7	
44	4 Wait	- 8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
45	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
46	91 \$15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
47	13 \$15 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
48	10 \$18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
49	4 Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
50	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
51	9 \$18 To Column	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
52	34 Flush to Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
53	9 \$18 To Column	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
54	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
55	9 \$18 To Column	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
56	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
57	1 Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
58	33 Cycle Entry	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
59	9 \$18 To Column	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
60	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
61	6 Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
62	30 \$17 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
63	11 \$17 To Column	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
64	34 Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
65	11 \$17 To Column	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
66	34 Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
67	11 \$17 To Column	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
68	34 Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
69	11 \$17 To Column	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
70	34 Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
71	11 \$17 To Column	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
72	34 Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
73	11 \$17 To Column	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
74	34 Flush to Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
75	9 \$18 To Column	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
76	34 Flush to Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
77	7 Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
78	9 \$18 To Column	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
79	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
80	9 \$18 To Column	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
81	2 Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
82	1 Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

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[illegible]

[illegible]

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STEP NUMBER	FUNCTION # NAME	STEP TIME	STEP ACTIVE FOR BASES							SAFE STEP
			A	G	C	T	S	6	7	
1	28 Phos Prep	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	52 A To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	53 G To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	54 C To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	55 T To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	56 #5 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	57 #6 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	58 #7 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	61 TET To Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	10 #18 To Waste	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	16 Cap Prep	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	53 Cap A To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	60 Cap B To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	81 #15 To Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	82 #14 To Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	30 #17 To Waste	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	10 #18 To Waste	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	1 Block Flush	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

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5'- GGT GTT TGG TTG TTG TTG TTG TTG TTG TTG TTG

TTG TTG TTG TTG TTG TTT TTT TTT TTT TTT TT -3'

DNA SEQUENCE  
VERSION 2.00

SEQUENCE NAME: 15X-2  
SEQUENCE LENGTH: 10  
DATE: Aug 27, 199  
TIME: 14:06  
COMMENT:

5'- TTT GAC TGG T -3'

Claims

1. A synthetic oligonucleotide useful as an amplifier probe in a sandwich hybridization assay for HBV comprising

5 a first segment comprising a nucleotide sequence substantially complementary to a segment of HBV nucleic acid; and

10 a second segment comprising a nucleotide sequence substantially complementary to an oligonucleotide multimer;

wherein said HBV nucleic acid segment is selected from the group consisting of

15 TTGTGGGTCTTTTGGGYTTTGCTGCTGCCWT (SEQ ID NO:6),  
CCTKCTCGTGTTACAGGCGGGTCTTTCTT (SEQ ID NO:7),  
TCCATGGCTGCTAGGSTGTRCTGCCAACTG (SEQ ID NO:8),  
GCTAYAGACCACCAAATGCCCCTATCYTA (SEQ ID NO:9),  
CTGTTCAAGCCTCCAAGCTGTGCCTTGGGT (SEQ ID NO:10),  
20 CATGGAGARCAVMACATCAGGATTCCTAGG (SEQ ID NO:11),  
TCCTGGYTATCGCTGGATGTGTCTGCGGCGT (SEQ ID NO:12),  
GGCGCTGAATCCYCGGACGACCCBTCTCG (SEQ ID NO:13),  
CTTCGCTTCACCTCTGCACGTHGCATGGMG (SEQ ID NO:14),  
GGTCTSTGCCAAGTGTGCTGACGCAACC (SEQ ID NO:15),  
25 CCTKCGCGGGACGTCCTTTGTYTACGTCCC (SEQ ID NO:16),  
MCCTCTGCCTAATCATCTCWTGTWCATGTC (SEQ ID NO:17),  
CGACCACGGGGCGCACCTCTCTTACGCGG (SEQ ID NO:18),  
TGCCCAAGGTCTTACAYAAGAGGACTCTTG (SEQ ID NO:19),  
CGTCAATCTYCKCGAGGACTGGGGACCCTG (SEQ ID NO:20),  
ATGTTGCCCCGTTTGTCTCTAMTCCAGGA (SEQ ID NO:21),  
30 ATCTTCTTTRTTGGTTCTTCTGGAYTAYCAA (SEQ ID NO:22),  
ATCATMTTCCTCTTCATCCTGCTGCTATGC (SEQ ID NO:23),  
CAATCACTCACCAACCTCYTGTCTCTCCAAY (SEQ ID NO:24),  
GTGTCYTGGCCAAAATTGCGAGTCCCCAAC (SEQ ID NO:25),  
35 CTCGTGGTGGACTTCTCTCAATTTCTAGG (SEQ ID NO:26),



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GACAAGAATCCTCACAATACCRRCAGAGTCT (SEQ ID NO:27),  
TTTTGGGGTGGAGCCCKCAGGCTCAGGGCR (SEQ ID NO:28),  
CACCATATTCTTGGAACAAGAKCTACAGC (SEQ ID NO:29),  
ACACTTCCGGARACTACTGTTGTTAGACGA (SEQ ID NO:30),  
GTVTCTTTYGGAGTGTGGATTGCACTCCT (SEQ ID NO:31),  
TTGGAGCWWCTGTGGAGTTACTCTCKTTTT (SEQ ID NO:32),  
TTTGGGGCATGGACATYGAYCCKTATAAAG (SEQ ID NO:33),  
AAWGRTCTTTGTAYTAGGAGGCTGTAGGCA (SEQ ID NO:34),  
RGACTGGGAGGAGYTGGGGGAGGAGATTAG (SEQ ID NO:35),  
CCTTGAGGCMTACTTCAAAGACTGKTGT (SEQ ID NO:36),  
GTCTGTGCCTTCTCATCTGCCGGWCCGTGT (SEQ ID NO:37),  
AGCMGCTTGTTTTGCTCGCAGSMGGTCTGG (SEQ ID NO:38),  
GGCTCSTCTGCCGATCCATACTGCGGAAC (SEQ ID NO:39),  
MTKAACCTTTACCCCGTTGCTCGGCAACGG (SEQ ID NO:40),  
GTGGCTCCAGTTCMGGAAACAGTAAACCCTG (SEQ ID NO:41),  
KAARCAGGCTTTYACTTTCTCGCCAACCTA (SEQ ID NO:42),  
CCTCCKCCTGCCTCYACCAATCGSCAGTCA (SEQ ID NO:43),  
ACCAATTTTCTTYTGTCTYTGGGTATACAT (SEQ ID NO:44).

2. The synthetic oligonucleotide of claim 1,  
wherein said second segment comprises

AGGCATAGGACCCGTGTCTT (SEQ ID NO:54).

3. A synthetic oligonucleotide useful as a  
capture probe in a sandwich hybridization assay for HBV  
comprising

a first segment comprising a nucleotide  
sequence substantially complementary to a segment of HBV  
nucleic acid; and

a second segment comprising a nucleotide  
sequence substantially complementary to an  
oligonucleotide bound to a solid phase,

wherein said HBV nucleic acid segment is  
selected from the group consisting of

TATTTCCCATCCCATCRTCCTGGGCTTTTCGS (SEQ ID NO:45),  
TATATGGATGATGTGGTATTGGGGGCAAG (SEQ ID NO:46),  
CGTAGGGCTTTCCCCCACTGTTTGGCTTTC (SEQ ID NO:47),  
GCTCAGTTTACTAGTGCCATTTGTTCAGTG (SEQ ID NO:48),  
5 CCTATGGGAGKGGGCCTCAGYCCGTTTCTC (SEQ ID NO:49),  
GTCCCCTAGAAGAAGAACTCCCTCGCCTCG (SEQ ID NO:50),  
ACGMAGRTCTCMATCGCCGCGTCGCAGAAGA (SEQ ID NO:51),  
CAATCTCGGGAATCTCAATGTTAGTATYCC (SEQ ID NO:52),  
GACTCATAAGGTSGGRAACTTTACKGGGCT (SEQ ID NO:53).

10 4. The synthetic oligonucleotide of claim 3,  
wherein said second segment is

CTTCTTTGGAGAAAGTGGTG (SEQ ID NO:55).

15 5. A set of synthetic oligonucleotides useful  
as amplifier probes in a sandwich hybridization assay for  
HBV, comprising two oligonucleotides, wherein each member  
of the set comprises

20 a first segment comprising a nucleotide  
sequence substantially complementary to a segment of HBV  
nucleic acid; and

a second segment comprising a nucleotide  
sequence substantially complementary to an  
oligonucleotide multimer;

25 wherein said HBV nucleic acid segments are

TTGTGGGTCTTTTGGGYTTTGCTGCGCCWT (SEQ ID NO:6),  
CCTKCTCGTGTTACAGGCGGGGTTTTTCTT (SEQ ID NO:7),  
TCCATGGCTGCTAGGSTGTRCTGCCAACTG (SEQ ID NO:8),  
30 GCYTAYAGACCACCAAATGCCCCTATCYTA (SEQ ID NO:9),  
CTGTTCAAGCCTCCAAGCTGTGCCTTGGGT (SEQ ID NO:10),  
CATGGAGARCAYMACATCAGGATTCCTAGG (SEQ ID NO:11),  
TCCTGGYTATCGCTGGATGTGTCTGCGGCGT (SEQ ID NO:12),  
GGCGCTGAATCCYCGGACGACCCBTCTCG (SEQ ID NO:13),  
35 CTTGCTTCACCTCTGCACGTHGCATGGMG (SEQ ID NO:14),

5 GGTCTSTGCCAAGTGTTTGCTGACGCAACC (SEQ ID NO:15),  
CCTKCGCGGGACGTCCTTTGTYTACGTCCC (SEQ ID NO:16),  
MCCTCTGCCTAATCATCTCWTGTWCATGTC (SEQ ID NO:17),  
CGACCACGGGGCGCACCTCTCTTTACGCGG (SEQ ID NO:18),  
TGCCCAAGGTCTTACAYAAGAGGACTCTTG (SEQ ID NO:19),  
CGTCAATCTYCKCGAGGACTGGGGACCCTG (SEQ ID NO:20),  
ATGTTGCCCGTTTGTCCTCTAMTTCCAGGA (SEQ ID NO:21),  
ATCTTCTTRTTGGTTCTTCTGGAYTAYCAA (SEQ ID NO:22),  
ATCATMTTCCTCTTCATCCTGCTGCTATGC (SEQ ID NO:23),  
10 CAATCACTCACCAACCTCYTGTCTCCAAY (SEQ ID NO:24),  
GTGTCYTGGCCAAAATTCGCAGTCCCCAAC (SEQ ID NO:25),  
CTCGTGGTGGACTTCTCTCAATTTTCTAGG (SEQ ID NO:26),  
GACAAGAATCCTCACAATACCRCAAGTCT (SEQ ID NO:27),  
TTTGGGGTGGAGCCCKCAGGCTCAGGGCR (SEQ ID NO:28),  
15 CACCATATTCTTGGGAACAAGAKCTACAGC (SEQ ID NO:29),  
ACACTTCCGGARACTACTGTTGTTAGACGA (SEQ ID NO:30),  
GTVTCTTTYGGAGTGTGGATTGCACTCCT (SEQ ID NO:31),  
TTGGAGCWWCTGTGGAGTTACTCTCKTTTT (SEQ ID NO:32),  
TTTGGGGCATGGACATYGAYCCKTATAAAG (SEQ ID NO:33),  
20 AAWGRTCTTTGTAYTAGGAGGCTGTAGGCA (SEQ ID NO:34),  
RGACTGGGAGGAGYTGGGGGAGGAGATTAG (SEQ ID NO:35),  
CCTTGAGGCMTACTTCAAAGACTGKTGTGTT (SEQ ID NO:36),  
GTCTGTGCCTTCTCATCTGCCGGWCCGTGT (SEQ ID NO:37),  
AGCMGCTTGTTTGTCTCGCAGSMGGTCTGG (SEQ ID NO:38),  
25 GGCTCSTCTGCCGATCCATACTGCGGAACT (SEQ ID NO:39),  
MTKAACCTTTACCCCGTTGCTCGGCAACGG (SEQ ID NO:40),  
GTGGCTCCAGTTCMGAACAGTAAACCCTG (SEQ ID NO:41),  
KAARCAGGCTTTYACTTTCTCGCCAACCTTA (SEQ ID NO:42),  
CCTCCKCCTGCCTCYACCAATCGSCAGTCA (SEQ ID NO:43),  
30 ACCAATTTTCTTYTGTCTYTGGGTATACAT (SEQ ID NO:44).

6. The set of synthetic oligonucleotides of claim 5, wherein said second segment comprises

35 AGGCATAGGACCCGTGTCTT (SEQ ID NO:54).

7. A set of synthetic oligonucleotides useful as capture probes in a sandwich hybridization assay for HBV, comprising two oligonucleotides, wherein each member of the set comprises

5 a first segment comprising a nucleotide sequence substantially complementary to a segment of HBV nucleic acid; and

a second segment comprising a nucleotide sequence substantially complementary to an oligonucleotide bound to a solid phase,

10 wherein said HBV nucleic acid segments are  
TATTCCCATCCCATCRTCCTGGGCTTTTCGS (SEQ ID NO:45),  
TATATGGATGATGTGGTATTGGGGGCCAAG (SEQ ID NO:46),  
CGTAGGGCTTTCCCCCACTGTTTGGCTTTC (SEQ ID NO:47),  
15 GCTCAGTTTACTAGTGCCATTTGTTTCAGTG (SEQ ID NO:48),  
CCTATGGGAGKGGGCCTCAGYCCGTTTCTC (SEQ ID NO:49),  
GTCCCCTAGAAGAAGAACTCCCTCGCCTCG (SEQ ID NO:50),  
ACGMAGRTCTCMATCGCCGCGTCGCAGAAGA (SEQ ID NO:51),  
CAATCTCGGGAATCTCAATGTTAGTATYCC (SEQ ID NO:52),  
20 GACTCATAAGGTSGGAACTTTACKGGGCT (SEQ ID NO:53).

8. The set of synthetic oligonucleotides of claim 7, wherein said second segment comprises  
CTTCTTTGGAGAAAGTGGTG (SEQ ID NO:55).

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9. A solution sandwich hybridization assay for detecting the presence of HBV in a sample, comprising

(a) contacting the sample under hybridizing conditions with an excess of (i) amplifier probes  
30 comprising the of set of synthetic oligonucleotides of claim 5 and (ii) a set of capture probe oligonucleotides wherein the capture probe oligonucleotide comprises a first segment comprising a nucleotide sequence that is substantially complementary to a segment of HBV nucleic  
35 acid and a second s gment that is substantially

complementary to an oligonucleotide bound to a solid phase;

(b) contacting the product of step (a) under hybridizing conditions with said oligonucleotide bound to the solid phase;

(c) thereafter separating materials not bound to the solid phase;

(d) contacting the bound product of step (c) under hybridization conditions with the nucleic acid multimer, said multimer comprising at least one oligonucleotide unit that is substantially complementary to the second segment of the amplifier probe polynucleotide and a multiplicity of second oligonucleotide units that are substantially complementary to a labeled oligonucleotide;

(e) removing unbound multimer;

(f) contacting under hybridizing conditions the solid phase complex product of step (e) with the labeled oligonucleotide;

(g) removing unbound labeled oligonucleotide; and

(h) detecting the presence of label in the solid phase complex product of step (g).

10. A solution sandwich hybridization assay for detecting the presence of HBV in a sample, comprising

(a) contacting the sample under hybridizing conditions with an excess of (i) a set of amplifier probe oligonucleotides wherein the amplifier probe oligonucleotide comprises a first segment comprising a nucleotide sequence substantially complementary to a segment of HBV nucleic acid and a second segment comprising a nucleotide sequence substantially complementary to an oligonucleotide unit of a nucleic acid multimer and (ii) capture probes

comprising the set of synthetic oligonucleotides of claim 7;

(b) contacting the product of step (a) under hybridizing conditions with said oligonucleotide bound to the solid phase;

(c) thereafter separating materials not bound to the solid phase;

(d) contacting the bound product of step (c) under hybridization conditions with the nucleic acid multimer, said multimer comprising at least one oligonucleotide unit that is substantially complementary to the second segment of the amplifier probe polynucleotide and a multiplicity of second oligonucleotide units that are substantially complementary to a labeled oligonucleotide;

(e) removing unbound multimer;

(f) contacting under hybridizing conditions the solid phase complex product of step (e) with the labeled oligonucleotide;

(g) removing unbound labeled oligonucleotide; and

(h) detecting the presence of label in the solid phase complex product of step (g).

11. A kit for the detection of HBV in a sample comprising in combination

(i) a set of amplifier probe oligonucleotides wherein the amplifier probe oligonucleotide comprises a first segment comprising a nucleotide sequence substantially complementary to a segment of HBV nucleic acid and a second segment comprising a nucleotide sequence substantially complementary to an oligonucleotide unit of a nucleic acid multimer;

(ii) a set of capture probe oligonucleotides wherein the capture probe oligonucleotide comprises a

first segment comprising a nucleotide sequence that is substantially complementary to a segment of HBV DNA and a second segment that is substantially complementary to an oligonucleotide bound to a solid phase;

- 5 (iii) a nucleic acid multimer, said multimer comprising at least one oligonucleotide unit that is substantially complementary to the second segment of the amplifier probe polynucleotide and a multiplicity of second oligonucleotide units that are substantially  
10 complementary to a labeled oligonucleotide; and  
(iv) a labeled oligonucleotide.

12. The kit of claim 11, wherein said  
15 amplifier probe oligonucleotide comprises the set of synthetic oligonucleotides of claim 5.

13. The kit of claim 11, wherein said capture  
20 probe oligonucleotide comprises the set of synthetic oligonucleotides of claim 7.

14. The kit of claim 10, further comprising  
instructions for the use thereof.

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US92/11165

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07H 21/02, 21/04; C12Q 1/68, 1/70

US CL :536/24.3; 435/5, 6

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/24.3; 435/5, 6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GENE, Vol. 30, issued 1984, M. Kobayashi et al, "Complete nucleotide sequence of hepatitis B virus DNA of subtype adr and its conserved gene organization", pages 227-232, see Figure 1.	1-14
Y	EP, A, 0,317,077 (URDEA ET AL) 24 MAY 1989, see entire document.	1-14
Y	WO, A, 89/03891 (URDEA ET AL) 05 MAY 1989, see entire document.	1-14
Y	US, A, 4,868,105 (URDEA ET AL) 19 SEPTEMBER 1989, see entire document, especially Figure 1A.	1-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	* T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
* A* document defining the general state of the art which is not considered to be part of particular relevance	* X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
* E* earlier document published on or after the international filing date	* Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
* L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	* &	document member of the same patent family
* O* document referring to an oral disclosure, use, exhibition or other means		
* P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

24 March 1993

Date of mailing of the international search report

30 MAR 1993

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. NOT APPLICABLE

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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US92/11165

## B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

N-GENESEQ, UEMBL, EMBL-NEW, GENBANK, GENBANK-NEW, MEDLINE, CAS, BIOSIS,  
search terms: HBV, hepatitis B virus, amplifier probe, multimer, sandwich hybridization, solid phase, nucleic acid,  
synthetic oligonucleotides

